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Regulation of the amyloid precursor protein ectodomain shedding by the 5-HT_4 receptor and Epac

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Abstract The serotonin 5-hydroxytryptamine (5-HT_4) receptor is of potential interest for the treatment of Alzheimer's disease because it increases memory and learning. In this study, we investigated the effect of zinc metalloprotease inhibitors on the amyloid precursor protein (APP) processing induced by the serotonin 5-HT_4 receptor in vitro. We show that secretion of the non-amyloidogenic form of APP, sAPPα induced by the 5-$HT_{4(e)}$ receptor isoform was not due to a general boost of the constitutive secretory pathway but rather to its specific effect on α-secretase activity. Although the h5-$HT_{4(e)}$ receptor increased IP3 production, inhibition of PKC did not modify its effect on sAPPα secretion. In addition, we found that α secretase activity is regulated by the cAMP-regulated guanine nucleotide exchange factor, Epac and the small GTPase Rac.

Keywords: Serotonin; Alzheimer's disease; Amyloid; Small G protein; cAMP; G protein-coupled receptor

1. Introduction

The amyloid precursor protein (APP) is a type I integral membrane glycoprotein constitutively expressed in many types of mammalian cells. APP is the precursor of the amyloid β peptide (Aβ), the main component of the senile plaques found in brains of Alzheimer's disease (AD) patients [reviewed in [1,2]]. Cleavage of APP at the N-terminus of Aβ by β-secretase and at the C-terminus by γ-secretase constitutes the amyloidogenic pathway and yields the Aβ peptide which easily aggregates in the brain [reviewed in [1]]. Alternatively, the α-secretase pathway cleaves APP in its extracellular domain and releases a large soluble N-terminal ectodomain named sAPPα into the extracellular space. The α cleavage is of physiological interest because secreted sAPPα has neuroprotective properties and potent memory-enhancing effects [reviewed in [3]].

Three members of the ADAM family (ADAM stands for a disintegrin and metalloprotease), ADAM-9, ADAM-10 and TACE (tumour necrosis factor α converting enzyme, ADAM17) have been identified as candidates for the α-secretase activity of APP [reviewed in [4]]. These metalloproteases are membrane-anchored proteins involved in the proteolytic cleavage or "shedding" of many transmembrane protein ectodomains [6]. They are inhibited by hydroxamic-acid-based zinc metalloproteases such as batimastat, marimastat, and tumour necrosis factor-α protease inhibitor (TAPI) [5,6]. The α-secretase cleavage occurs constitutively in all cell lines examined but can be regulated by G protein-coupled receptors (GPCRs) [7,8]. Many studies have explored the role of protein kinase C (PKC) in APP metabolism and there are now some evidence that constitutive and regulated α-secretase cleavage of APP involved ADAM-10 and TACE [7]. To date, the effects of cAMP-dependent signalling on the shedding of APP ectodomain have not yet been investigated.

The 5-hydroxytryptamine (5-HT_4) receptor has gained a lot of attention for its physiological effects on the brain [9]. This receptor is positively coupled to adenylyl cyclase and activates the cAMP-regulated guanine nucleotide exchange factor (GEF), Epac through the small GTPases Rap1 and Rac [10]. Pharmacological studies have shown that activation of the 5-HT_4 receptor increases memory and learning in rats [9,11]. Further support for a therapeutic potential of 5-HT_4 receptor agonists in the treatment of AD comes from our observations that the 5-HT_4 receptor can regulate APP processing [12]. To further understand the influence of the 5-HT_4 receptor in this process, we have examined the effects of a number of metalloprotease inhibitors on basal and 5-HT_4 receptor-regulated sAPPα release. Experiments were carried out in Chinese hamster ovary (CHO) cells stably expressing the h5-$HT_{4(e)}$ receptor, a neuronal 5-HT_4 receptor isoform. In addition, we analysed the effects of various cAMP analogues as well as Epac

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Abbreviations: ACh, acetylcholine; AD, Alzheimer's disease; ADAM, a disintegrin and metalloprotease; Aβ, amyloid β-peptide; APP, amyloid precursor protein; sAPPα, soluble form of the amyloid precursor protein; FSK, forskolin; GEF, guanine nucleotide exchange factor; GPCRs, G protein-coupled receptors; 5-HT, 5-hydroxytryptamine; h5-HT_4, human 5-HT_4 receptor; IP3, inositol 1,4,5-trisphosphate; FCS, foetal calf serum; CHO cells, Chinese hamster ovary cells; SDS, sodium dodecyl sulfate; PKC, protein kinase C; SEAP, secreted placental alkaline phosphatase; TACE, tumour necrosis factor α converting enzyme; ADAM17; TAPI, tumour necrosis factor-α protease inhibitor

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and Rac activation on APP ectodomain shedding. Aβ production was measured upon 5-HT_4 receptor and Epac activation.

2. Materials and methods

2.1. Materials

All media, sera, and antibiotics used in the cell culture were purchased from Life Technologies (Cergy Pontoise, France). The 5-HT_4 agonist, cisapride was synthesised in Dr. Sam Sicsic's laboratory (BIOCIS, CNRS, Châtenay-Malabry, France). The 5-HT_4 agonist, renzapride, the 5-HT_4 receptor antagonist, GR113808, as well as the 5-HT_{1B} receptor antagonist GR127935 were generously given by GlaxoSmithKline (Harlow, UK). The 5-HT_4 agonist, prucalopride was a gift from Janssen Research Foundation (Beerse, Belgium). 5-HT was from Aldrich (L'Isle d'Abeau Chesnes, France). 8-(4-Chloro-phenylthio)-2′-*O*-methyladenosine-3′-5′-cyclic monophosphate (8-pCPT-2′-O-Me-cAMP) was from Biolog Life Science Institute (Bremen, Germany). BIM1, phorbol 12-myristate 13-acetate (PMA), forskolin (FSK) and 8-bromo-cyclic AMP (8-bromo-cAMP) were obtained from Calbiochem (France Biochem, Meudon, France). TAPI-2 was given by Amgen (Thousand Oaks, CA). Marimastat and batimastat were provided by GlaxoSmithKline (Harlow, UK).

2.2. Cell culture and transient transfection

CHO cells stably coexpressing the h5-$HT_{4(e)}$ receptor isoform and APP695 were cultured as previously described [8]. Transient transfection experiments were performed using jetPEI™ (Polyplus-transfection, Illkirch, France) according to the manufacturer's instructions.

2.3. Measurement of sAPPα by Western blot

For determination of secreted sAPPα, confluent CHO and IMR32 cell cultures were cultured in 5% dialysed foetal calf serum (FCS)-containing medium overnight and in serum free medium for 4 h, respectively. After drug treatment in serum free medium, media were centrifuged to remove cell debris and concentrated by ultrafiltration (Vivaspin columns, Vivascience, Palaiseau, France). Collected media were reconstitued in 70 μl of buffer consisting of 50 mM Tris, pH 7.4, 5 mM EDTA, and 1 mM phenylmethylsulfonyl fluoride. The protein amount in each sample was determined with the bicinchoninic acid assay (Sigma, Saint Quentin Fallavier, France). 70 μg of secreted proteins were run on 8% sodium dodecyl sulfate (SDS) polyacrylamide gel and secreted sAPPα was detected as previously described [12] by Western blotting using the monoclonal antibody 6E10 (Signet Pathology Systems, Dedham, USA) which is specific for the α-secretase cleaved form of human APP. For quantification, films of representative experiments were scanned using a Biorad image acquisition system (Ivry-Sur-Seine, France, gel doc 1000) and fold induction of sAPPα expression level compared to corresponding controls was calculated. Western blot experiments were repeated at least three times with identical results.

2.4. Secreted placental alkaline phosphatase (SEAP) assay

For determination of SEAP activity, CHO cells stably expressing the h5-$HT_{4(e)}$ receptor isoform were cultured in 96 well-plates. Twenty four hours after transfection with a recombinant vector encoding the human SEAP, confluent cells were washed with serum-free medium and preincubated for 10 min in serum-free medium supplemented with 1 μM GR127935 to block the activity of endogenous 5-HT_{1B} receptor, and increasing concentrations of 5-HT_4 ligands were added for 30 min. Conditioned supernatants were collected and heated at 65 °C for 5 min. Five μl of supernatant was added to 100 μl of phosphatase substrate solution (Sigma, Saint Quentin Fallavier, France) for an incubation of 30 min at 37 °C. SEAP activity was determined by colorimetric assay at 450 nm.

2.5. Determination of Aβ

Two days after plating, cells were cultured in 5% dialysed FCS-containing medium for an overnight period. Then, cells were washed with serum-free medium, and new fresh medium was added. Cells were treated with different concentrations of 5-HT, or prucalopride, or vehicle at the beginning of the assay and 24 hs later. After this 48-h period, conditioned media were collected and frozen at −80 °C until ELISA analysis. Total Aβ was detected by a electrochemiluminescence assay with an Origen M8 Analyzer (IGEN Europe Inc., Oxford, UK). The 4G8 antibody (Senetek PLC, Napa, CA) directed against the Aβ 17–24 sequence was ruthenylated with TAG-NHS ester according to the supplier's instructions (IGEN Europe Inc.) and used in conjunction with the 6E10-biotinylated antibody (Senetek PLC) directed against the Aβ 5–15 sequence.

2.6. IP3 measurement

Confluent CHO cell cultures were cultured in 5% dialysed FCS-containing medium overnight. At the beginning of the assay, CHO cells were washed with serum-free medium and preincubated for 10 min in serum-free medium supplemented with 1 μM GR127935. Then, 5-HT_4 ligands and drugs were added for the indicated time periods. Upon stimulation, cells were scraped in cold phosphate buffer solution and immediately frozen at −80 °C until inositol 1,4,5-trisphosphate (IP3) was measured using a tritiated radioimmunological kit (Amersham Biosciences, Les Ulis, France).

2.7. Statistical analysis

An unpaired Student's *t*-test was used to calculate differences between means; differences were considered significant when P was <0.05.

3. Results

The effects of three inhibitors of zinc metalloproteases, batimastat, marimastat, and TAPI on the activity of α-secretase were examined in CHO cells stably coexpressing the h5-$HT_{4(e)}$ receptor isoform and APP695. When CHO cells were treated with batimastat (10 μM), marimastat (10 μM) or TAPI (10 μM), activation of the 5-HT_4 receptor with 5-HT (1 μM) or prucalopride (1 μM) failed to increase sAPPα release (Fig. 1A and B). Similar results with prucalopride were obtained in human neuroblastoma IMR32 cells, which express endogenous h5-HT_4 receptors (human 5-HT_4 receptor, data not shown). The contribution of metalloprotease in the α cleavage of APP induced by the 5-HT_4 receptor was also investigated by transfecting ADAM10 constructs. As shown in Fig. 1C, an inactive form of ADAM10 (ADAM10-DN) [13] inhibited 5-HT-induced secretion of sAPPα whereas transfection of the wild-type form of ADAM10 (ADAM-WT) mimicked the stimulating effect of 5-HT on sAPPα secretion (Fig. 1C). Similar levels of sAPPα were detected in CHO cells transfected with ADAM-WT in the absence or in the presence of 5-HT (Fig. 1C), probably because the α cleavage of APP reached its maximum upon tranfection of ADAM-WT. Moreover, the fact that ADAM10-DN enhanced sAPPα secretion compared to mock-transfected cells may be explained by its residual catalytic activity. Altogether these results show that induced sAPPα shedding by 5-HT_4 receptor involves zinc metalloproteases. Next, we investigated whether the constitutive secretory pathway may be affected by the 5-HT_4 receptor. To do this, we used SEAP which is known to be a marker for the constitutive secretory pathway [14]. Fig. 1D shows that 5-HT did not significantly alter SEAP activity. This result indicates that the influence of the 5-HT_4 receptor on APP processing is due to a specific effect on α-secretase activity rather than a general boost of the constitutive secretory pathway.

All the C-terminal splice variants of the h5-HT_4 receptor are positively coupled to adenylyl cyclase [15]. Besides cAMP, we found here that activation of the 5-$HT_{4(e)}$ receptor isoform can also induce IP3 production. Indeed, increasing concentrations

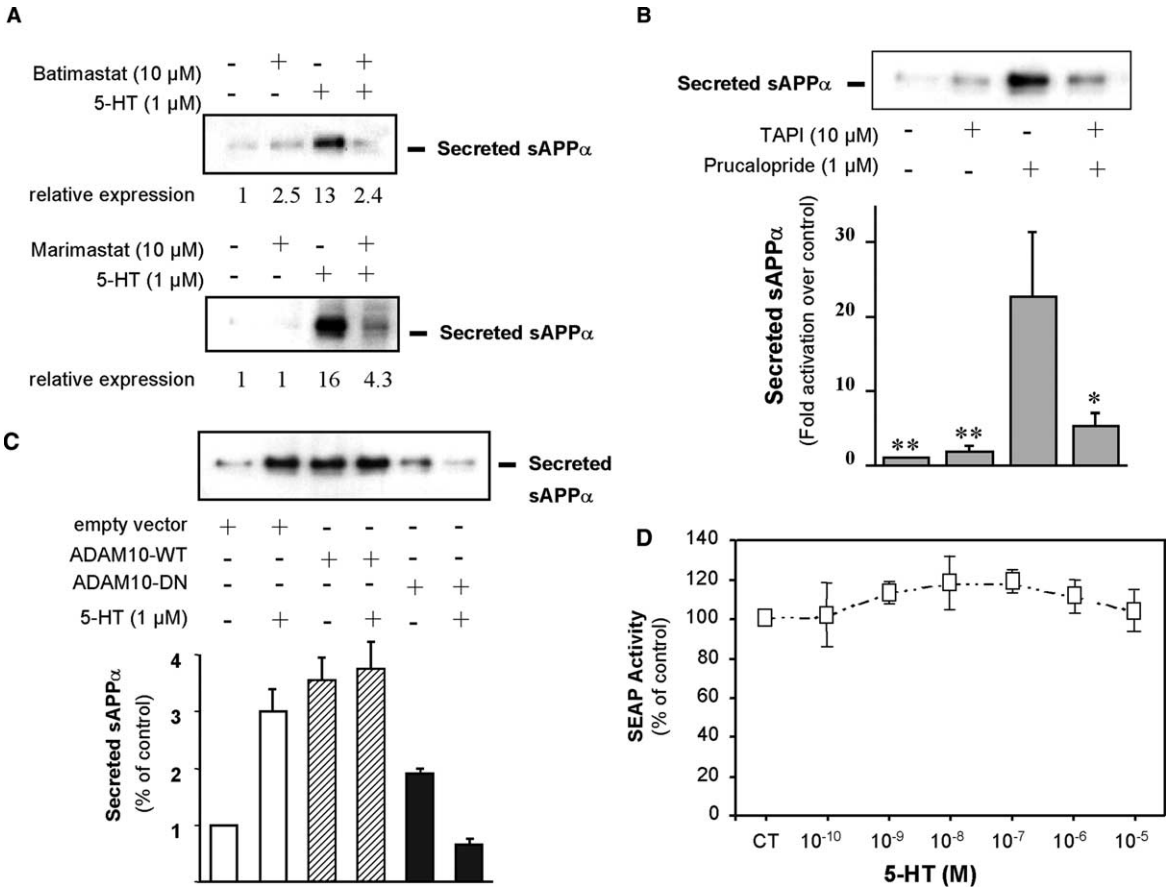

Fig. 1. Inhibition of 5-HT_4 receptor-induced sAPPα release by zinc metalloprotease inhibitors. (A) CHO cells stably expressing the h5-$HT_{4(e)}$ receptor isoform and the APP695 were pre-incubated with 10 μM of batimastat or marimastat just prior to treatment with 1 μM of 5-HT. After a 30-min period, sAPPα was detected in the culture medium by immunoblotting. (B) Effect of TAPI on prucalopride (1 μM) induced-sAPPα secretion. The amounts of secreted sAPPα detected in the media was expressed as fold activation over control. sAPPα expression in untreated control cells was defined arbitrarily as 1. Results are means ± S.E.M. for three independent experiments. $*P < 0.05$, $**P < 0.01$ compared with prucalopride treated cells (*t*-test). (C) An inactive form of ADAM10 inhibits 5-HT_4 receptor induced sAPPα secretion. CHO cells were transfected with the empty vector (8 μg), or ADAM-WT (8 μg) or ADAM-DN (8 μg). One day after, cells were stimulated with 1 μM 5-HT for 30 min and sAPPα secretion was determined as above. Upper panel, a representative Western blot is shown. Lower panel, the quantification of the signal is presented as relative protein expression. The expression in untreated control cells transfected with the empty vector was defined arbitrarily as 1. Results are means ± S.E.M. for two independent experiments. (D) 5-HT_4 receptor activation does not affect SEAP activity. CHO cells were transiently transfected with 8 μg of plasmid construct encoding the human SEAP. One day later, cells were treated with increasing concentration of 5-HT (10^{-10}–10^{-5} M) for 30 min and enzymatic activity was detected in the culture medium by colorimetric assay. Values were expressed as percentage of the control untreated cells in the same experiment. Results are means ± S.E.M. for two independent experiments performed in duplicates. CT, untreated control cells.

of 5-HT or prucalopride for 5 min enhanced IP3 production (Fig. 2A and B). Similarly, treatment of CHO cells with the partial 5-HT_4 agonists, renzapride (1 μM) or cisapride (1 μM), increased IP3 accumulation (Fig. 2B). Increase IP3 production is generally accompanied by diacylglycerol production which, concomitantly with the release of calcium from intracellular stores, activates PKC. Activation of PKC has been shown to favour α-secretase cleavage of APP and this process involved the ADAM family of metalloproteases [5,13,16,17]. Therefore, we analysed the potential involvement of PKC in 5-HT_4 receptor regulated α-secretase cleavage. Direct stimulation of PKC by the PMA (1 μM) strongly induced sAPPα secretion (Fig. 2C, lane 6). The lower migrating band that appears in PMA-treated cells may result from a different glycosylation state of secreted sAPPα (Fig. 2C, lane 6). Such a migration profile for sAPPα was observed in other works [12,17]. The PMA response as well as constitutive α-secretase cleavage of APP were completely blocked when cells were pre-treated with a pharmacological PKC inhibitor, BIM1 (10 μM) (Fig. 2C). However, BIM1 failed to block the ability of 5-HT to stimulate sAPPα secretion (Fig. 2C). Therefore, although the 5-HT_4 receptor enhanced IP3 production, PKC is not involved in 5-HT_4 receptor-induced α-secretase cleavage of APP.

Next, we focused our study on the involvement of cAMP signalling pathway in APP ectodomain shedding. An activator of adenylyl cyclase, FSK (10 μM), or with 8-Br-cAMP (10 μM) mimicked the effects of 5-HT since they caused an increase in the release of sAPPα (Fig. 3A and B). cAMP-induced sAPPα release was inhibited with batimastat (10 μM) (Fig. 3A), or TAPI (10 μM) (Fig. 3B), or marimastat (data not shown). This result indicates that ADAM proteases contribute to cAMP-regulated α-secretase cleavage of APP. We have recently shown that the 5-HT_4 receptor activates a new signalling pathway that involves the cAMP-regulated GEF, Epac and the

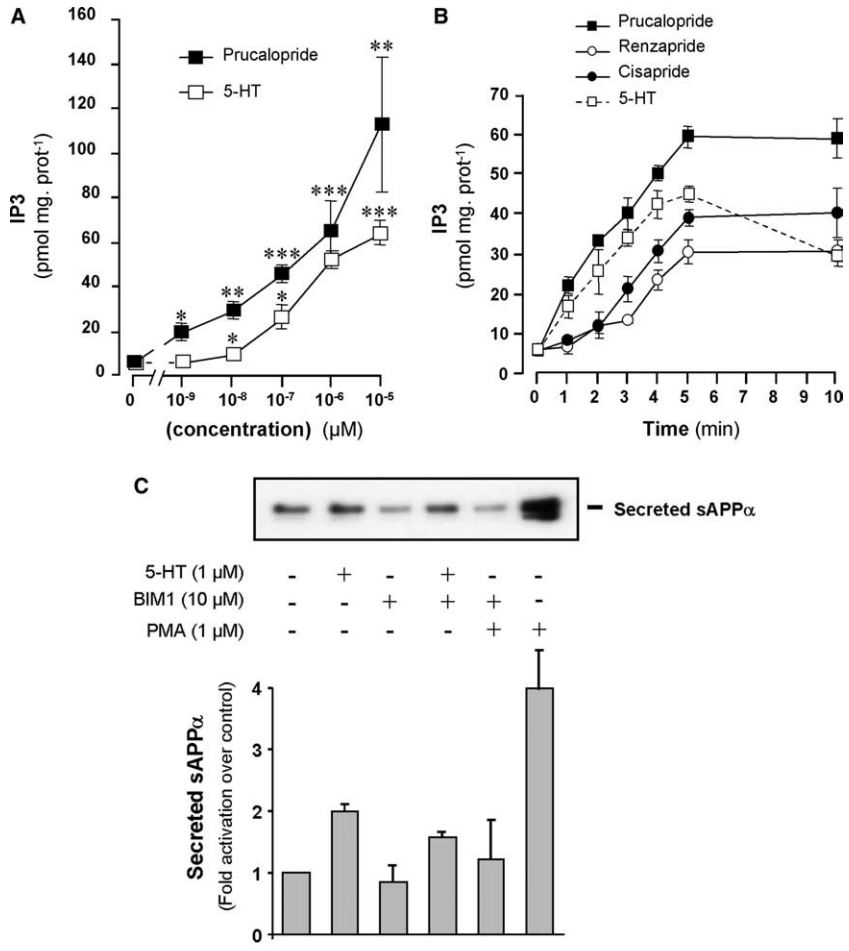

Fig. 2. 5-HT_4 receptor activation enhanced IP3 production but PKC is not involved in 5-HT_4 receptor-induced sAPPα secretion. (A) Concentration-dependent and (B) time-dependent stimulation of IP3 production upon activation of the 5-$HT_{4(e)}$ receptor with various agonists. (A) CHO cells were treated with increasing concentrations of either 5-HT or prucalopride for 5 min. Data are means ± S.E.M of three experiments performed in triplicates. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with control cells (*t*-test). (B) Differences were statistically significant upon 1 min treatment with 5-HT or prucalopride ($P < 0.01$ and $P < 0.001$), or 3 min treatment with renzapride or cisapride ($P < 0.05$, $P < 0.01$, and $P < 0.001$) compared to control cells. ■, prucalopride; ○, renzapride; ●, cisapride; and □, 5-HT. (C) Secreted sAPPα induced by the activation of the 5-$HT_{4(e)}$ receptor is not inhibited by a pharmacological inhibitor of PKC, BIM1. CHO cells were pre-incubated or not with 1 μM BIM1 for 10 min prior to treatment with 1 μM of 5-HT or PMA. After an additional period of 30 min, sAPPα was detected in the culture medium by immunoblotting. Upper panel, a representative immunoblot is shown. Lower panel, the amounts of secreted sAPPα detected in the media was expressed as fold activation over control. sAPPα expression in untreated control cells was defined arbitrarily as 1. Results are means ± S.E.M. for two independent experiments.

small GTPase Rac [10]. Therefore, we examined the involvement of Epac1 and Rac in APP ectodomain shedding. To do this, we used a Epac-specific cAMP analogue which does not activate cAMP-dependent protein kinase, 8-pCPT-2′-*O*-Me-cAMP [18]. As shown in Fig. 3A and B, 8-pCPT-2′-O-Me-cAMP (10 μM) strongly enhanced basal release of sAPPα which was completely blocked in the presence of 10 μM metalloprotease inhibitors. Similarly, transfection of CHO cells with a constitutive activated form of Epac1 (Epac-ΔcAMP) or Rac (Rac^{GV12}) increased the cellular release of sAPPα which was strongly inhibited in the presence of TAPI (10 μM) (Fig. 3C) or marimastat (10 μM) (data not shown).

In order to elucidate whether the stimulating effect of the 5-$HT_{4(e)}$ receptor isoform on sAPPα may also affect Aβ generation, total Aβ (Aβ40 and Aβ42) was assayed by *ELISA* in CHO cell culture media (Fig. 4A). We did not find any concentration-dependent effect of either 5-HT or prucalopride on extracellular Aβ compared to vehicle control CHO cells (Fig. 4A). As Epac is one of the main downstream component of the 5-$HT_{4(e)}$ receptor isoform which is involved in APP processing, we also assessed its effect on the production of extracellular Aβ. In contrast to what we observed on sAPPα release (Fig. 3), selective activation of Epac with 10 μM 8-pCPT-2′-*O*-Me-cAMP did not produce any significant change in the levels of extracellular Aβ compared to control saline-treated cells (Fig. 4B).

4. Discussion

We showed here that secretion of sAPPα induced by the h5-$HT_{4(e)}$ receptor was not due to a general boost of the constitutive secretory pathway. In contrast, the zinc metalloprotease inhibitors, batimastat, marimastat, and TAPI were effective

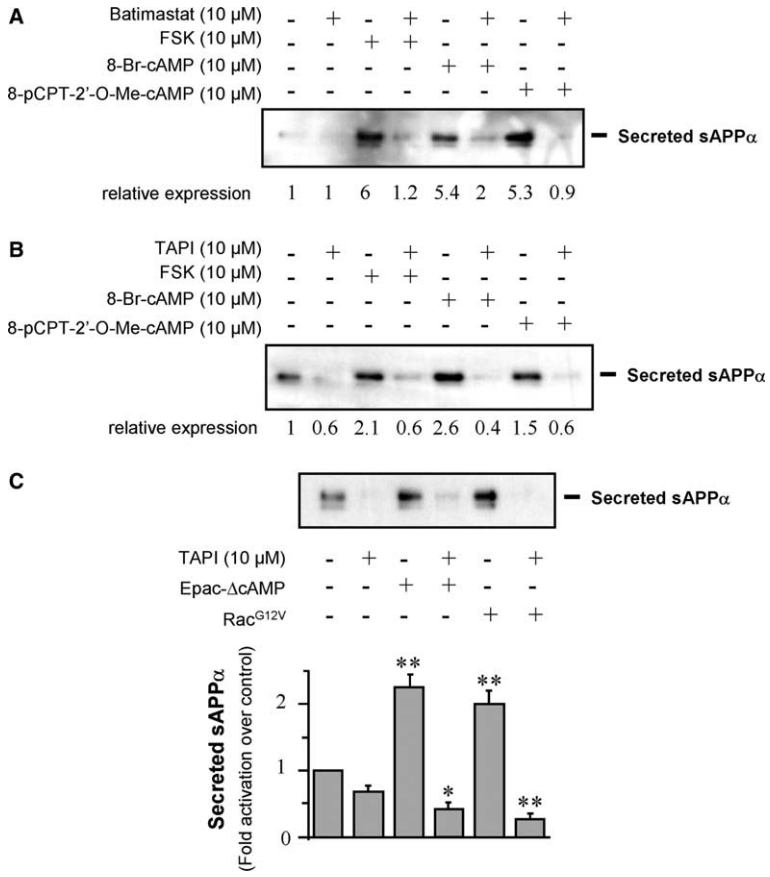

Fig. 3. cAMP-dependent activation of sAPPα secretion is inhibited by zinc metalloprotease inhibitors in CHO cells. cells were treated with FSK (10 μM), 8-Br-cAMP (10 μM) or 8-pCPT-2′-*O*-Me-cAMP (10 μM) for 30 min in the absence or presence batimastat (10 μM) (A) or TAPI (10 μM) (B). sAPPα release was detected in the culture medium by immunoblotting using the 6E10 antibody. (C) Epac1- and Rac-enhanced sAPPα release are inhibited by TAPI. CHO cells were transiently transfected with vector alone (8 μg), or constitutive activated forms of Epac (Epac-ΔcAMP) (8 μg), or Rac^{G12V} (8 μg). One day after, cells were treated or not with TAPI (10 μM) for 30 min and sAPPα secretion was assessed by Western blot. A representative immunoblot is shown. The expression in untreated control cells transfected with the empty vector was defined arbitrarily as 1. Results are means ± S.E.M. for three independent experiments. $*P < 0.05$, $**P < 0.01$, compared with control cells (*t*-test). Note that the differences observed in basal levels of sAPPα in control cells presented in (A), (B) and (C) may be explained by small differences in cell density between the experiments. Indeed, cell density has been recently reported to influence basal and regulated levels of sAPPα in several cell lines [29].

in blocking sAPPα release induced by the 5-HT$_4$ receptor indicating that the receptor regulates the activity of the α secretases. In our experiments, the metalloproteases inhibitors were added extemporaneously with 5-HT$_4$ ligands in cell culture media. Using this protocol, we were able to completely block 5-HT$_4$ receptor-induced sAPPα release. Therefore, the kinetic of activity of ADAM proteins upon 5-HT$_4$ receptor stimulation seems to parallel the activity of the receptor which reaches its maximun in terms of cAMP production and amount of Rac-GTP within 15 min [10]. Moreover, we found that exogenous ADAM-DN inhibited 5-HT- induced sAPPα secretion. Altogether, these data suggest the involvement of either one or more α-secretases in the regulation of APP ectodomain shedding induced by the 5-HT$_4$ receptor.

In addition to its coupling to Gs protein, we report here for the first time that the h5-HT$_{4(e)}$ receptor isoform is able to regulate IP3 production. Our results are in accordance with a previous finding showing that the h5-HT$_{4(b)}$ receptor isoform is coupled to Gαi/o proteins, in addition to its well-documented coupling to Gαs [19]. Such observation is not restricted to h5-HT$_4$ receptor isoforms since other GPCRs, such as C-terminal splice variants of the prostaglandin EP3, couple to different G proteins to activate different messenger systems [20]. The fact that a pharmacological inhibitor of PKC, BIM1 failed to block the effect of 5-HT$_4$ receptor activation on sAPPα secretion indicates that PKC is not involved in 5-HT$_4$ receptor-induced α-secretase cleavage of APP. However, this does not exclude the participation of PKC in the constitutive release of sAPPα since we observed a decrease in the constitutive secretion of sAPPα in the presence of BIM1 in CHO cells. Accordingly, the involvement of PKC in sAPPα constitutive secretion has also been observed in other cellular systems, such as the NT2N neuronal cell line [17].

Recently, we have proposed a new signalling pathway in which the small G protein Rac is activated by Rap1 following cAMP binding to the cAMP-GEF, Epac [10]. Rac belongs to the Rho family of GTPases which includes Rho, Rac and Cdc42 and is best known for its role in the modulation of the actin cytoskeleton [21]. Here we showed that Rac is implicated in a precise cellular function which involves the 5-HT$_4$ receptor and APP ectodomain shedding. Indeed, in this study, we found that the stimulating effect of FSK and cAMP analogues such as 8-Br-cAMP and 8-pCPT-2Me-cAMP on sAPPα cleavage was blocked by batimastat and TAPI. Transfection of

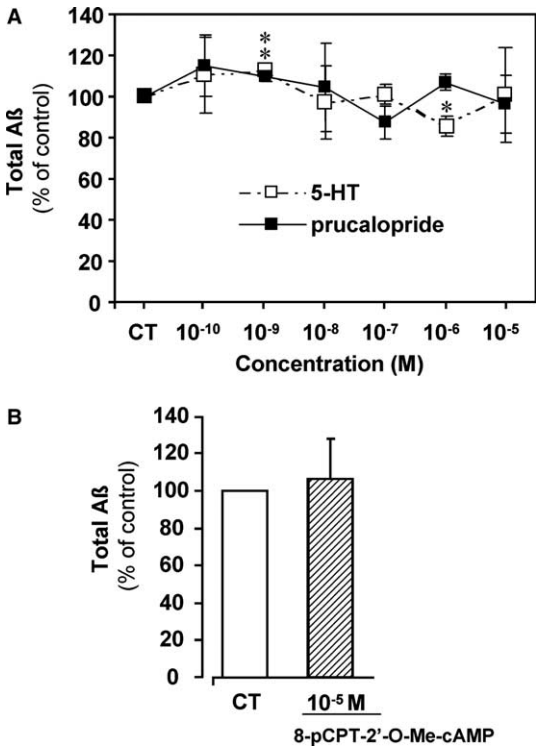

Fig. 4. Effect of h5-$HT_{4(e)}$ receptor and Epac activation on Aβ secretion. CHO cells were treated either with increasing concentrations of 5-HT_4 receptor agonists (5-HT or prucalopride) (A) or with 10 μ M of the specific activator of Epac, 8-pCPT-2′-*O*-Me-cAMP (B). Two days later, extracellular Aβ was assayed by ELISA. Aβ content was expressed as percentage of control cells (CT) (preincubated with saline vehicle only). Data are means ± S.E.M. of three independent experiments performed in quadruplets. $*P < 0.05$ compared with control cells (*t*-test).

dominant positive forms of Epac or Rac mimicked the effect of cAMP analogues on sAPPα secretion, and this was decreased with the metalloprotease inhibitors. Our results indicate that Epac and the small G protein Rac may influence α secretase activity. In accordance with our data, some metalloproteases albeit distinct from the ADAM family have been shown earlier to be regulated by Rac [22].

Our observation raises the question of how the activity of ADAM proteins is regulated by the small GTPase Rac. It is possible that ADAM activity is increased following phosphorylation by a kinase. Indeed, it has been shown that ADAM-9 is phosphorylated in vitro by purified PKC and in intact cells treated with phorbol esters [23,24]. With regards to the 5-HT_4 receptor signalling pathway, such a possible candidate could be the p21-Activated Kinase (PAK) which is a downstream effector of Rac. PAK has been shown to be required for processes involved in neurite formation and axonal guidance [25]. Interestingly, PAK3 has been recently isolated as an APP-interacting protein [26].

Another interesting aspect arising from this study, was the effect of the 5-HT_4 receptor and its associated signalling pathway on Aβ secretion. We did not observe any effect of 5-$HT_{4(e)}$ receptor and Epac activation on the extracellular levels of total Aβ. Accordingly, other studies failed to demonstrate any inverse correlation between sAPPα secretion and Aβ generation suggesting that the α- and β-secretase pathways may be differentially controlled [27]. Recent observations suggest that small G proteins may be potential candidates to differentially modulate the cleavage pattern of APP. Indeed, inhibition of the small GTPase Rho has been shown to lower preferentially the amount of Aβ42 [28]. Since, in our experimental conditions, total extracellular Aβ was quantified, we cannot exclude that Rac may differentially influence Aβ40 and Aβ42 levels. One could imagine that Rho and Rac have differential effects on APP metabolism. Rac could influence the activity of α-secretase whereas Rho would be devoted to the regulation of the γ-secretase activity. Therefore, potential therapeutic strategies for the treatment of AD might be directed to modulate the activity of the small G proteins such as Rho and Rac.

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